3. SAMPLING AND ANALYTICAL METHODS

3.1. Study Design and Study Questions

The Small Streams Toxicity Study was designed to answer the following questions:

- Is toxicity observed in small streams?
- If toxicity is observed, is it observed during different times of the year and during different hydrologic conditions (i.e., storm events, baseflow conditions)?
- If toxicity can be observed, to what extent can it be linked to pesticides or other toxicants?
- To answer the study questions the following tests and analyses were performed:
- Samples were tested for chronic toxicity to Ceriodaphnia dubia (water flea) and Selenastrum capricornutum (green algae). Samples were also analyzed for organophosphate pesticides, chlorinated herbicides, triazine herbicides, chlorinated insecticides, and glyphosate (Roundup™ is a common trade name). Metals, total organic carbon, and total suspended solids were also analyzed. The pesticide, metal, and conventional samples were collected concurrently with toxicity samples.
- Because toxicity observed in the streams may also be caused by other organic chemicals, some samples were analyzed for base/neutral/acid (BNA) extractable organic compounds. In past studies these organic chemicals have not been regularly detected in surface water samples, therefore only limited analysis of BNA organics were conducted.

To determine temporal patterns of toxicity, samples were collected at different times of year and under different hydrologic conditions. Four sampling events were chosen: during a spring storm, under summer baseflow conditions (non-storm), during an early fall storm, and during a late fall storm.

The timing of the spring and early fall sampling events was selected based on pesticide sales information. Sales data collected by King County from home improvement centers showed that the sale of lawn care products peaks in the spring, with a secondary peak in the fall (Market Trends Incorporated 1996). To determine if any observed toxicity was associated with baseflow conditions (i.e., not storm driven), samples were also collected in the summer during a period of dry weather and when application of pesticides would likely be low.

Finally, in order to evaluate potential differences in peak pesticide concentrations expected to occur as a result of seasonal variations in pesticide use and stormwater runoff, samples were also collected in the late fall. The late fall sampling event was selected to evaluate conditions associated with the period after the storm season had already begun. Pesticide application during this time had likely declined, and much of

the previously applied pesticides had presumably been transported off the landscape with the early fall storms.

3.2. Study Area

Sampling locations were selected based on surface water pesticide data collected by the USGS during the NAWQA synoptic pesticide survey in 1998 (Voss and Embrey 2000). The study area is shown in Figure 1.

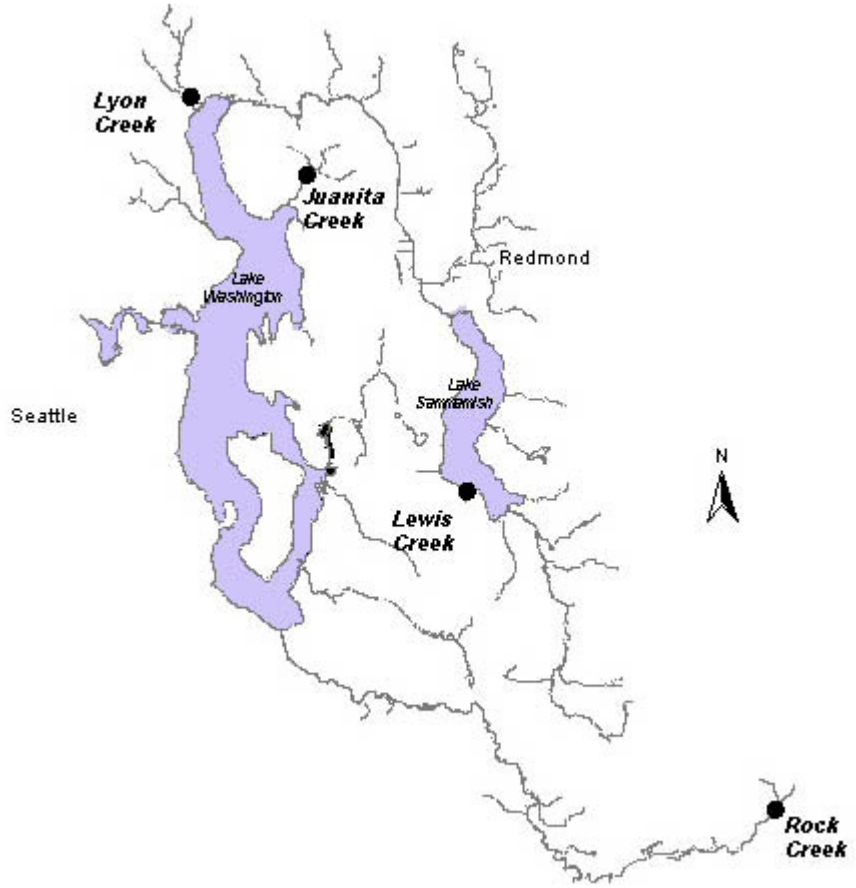

Figure 1. Study Area and Stream Locations

Site selection was intentionally biased towards the sites with the highest frequency of pesticide detections and the highest pesticide concentrations to increase the probability of observing toxicity and detecting contaminants that may be present. Two tests sites, Lyon and Juanita Creeks, were selected for spring, summer, fall, and late fall. Also based on the 1998 USGS pesticide survey results, an additional site, Lewis Creek, was sampled one-time in the spring. At Lewis Creek, the USGS pesticide survey detected lower concentrations of pesticides relative to the other streams tested during the survey (Voss and Embrey 2000). Lewis Creek was chosen to compare toxicity test results from a stream that showed relatively low pesticide concentrations to streams with relatively high pesticide concentrations (i.e., Lyon and Juanita). The reference site, Rock Creek, was sampled during every event for comparison to test sites.

3.2.1. Lyon Creek

The headwaters for Lyon Creek are located in the cities of Mountlake Terrace and Brier in Snohomish County. The creek then flows through Lake Forest Park before flowing into the North end of Lake Washington. The Lyon Creek drainage basin is approximately 3.7 square miles. Land use is primarily residential (66 percent). The average residential parcel size is 0.33 acres. A total of 4.7 percent of the landuse is commercial and 0.3 percent is industrial. The remaining land use covers 29 percent of the basin and is comprised of open space, parks, and forested cover.

3.2.2. Juanita Creek

The headwaters of Juanita Creek are located North of the City of Kirkland in unincorporated King County. The mainstem of the creek flows through Kirkland and Juanita Beach Park then empties into Lake Washington in Juanita Bay. The Juanita Creek drainage basin is approximately 7 square miles. Land use in the basin is primarily residential (54 percent). The average residential parcel size in 0.22 acre. Another 6 percent is commercial and 0.2 percent is classified as industrial. The remaining landuse covers 39 percent and is comprised of open space, parks, and forested cover.

3.2.3. Lewis Creek

The headwaters for Lewis Creek are located on the north slopes of Cougar Mountain. The Creek then flows through the Lakemont area of Bellevue before flowing into the southern end of Lake Sammamish. Land use in the Lewis Creek basin is 38 percent residential, 2.1 percent commercial, and 0.2 percent industrial. The remaining 59 percent of the land use is comprised of open space, parks, and forested cover. The average residential parcel size is 0.44 acre. Lewis Creek was chosen as a study site in part because the land use information indicated that the basin is fundamentally different than Lyon or Juanita. Much less of the Lewis Creek basin has been developed for residential use, and much more area is still forested. Also, the residential parcel size is larger than the other two basins.

3.2.4. Rock Creek

Rock Creek is located in an undeveloped forested basin within the Cedar River watershed within the boundaries of the City of Seattle watershed and was selected as the reference site.

3.3. Sample Collection

Sample collection methods are detailed in the Small Streams Toxicity Study Sampling and Analysis Plan (Appendix A). Sample collection was primarily conducted during storm events to assess runoff of toxicants. In addition, samples were also collected during summer baseflow conditions during a dry period in August. These samples were collected to compare the differences between storm flows and baseflows (see Table 2-1). However, due to budgetary constraints, Lewis Creek was only tested in the spring 1999 sampling event. Storm sampling was intended to capture the conditions at the rising stage of the stream during the storm. Studies have shown that some of the highest pesticide concentrations tend to be associated with the "first flush" or the initial wash of stormwater into surface waters (Williams 1998a, Williams 1998b). The Small Streams Toxicity/Pesticide study was intended to assess the peak concentration of potential toxicants in an attempt to define the upper limit of toxicity in the stream. Storm sampling was intended to commence when a storm of a high enough magnitude occurred (at least 1/4 to 1/2 inch of rain) and caused visible turbidity in the streams.

Table 2-1. 1999 sampling summary.

Site	Spring Runoff	Summer Baseflow	Early Fall Runoff	Late Fall Runoff
Lyon Creek	Toxicity, Pesticides, Metals, TSS, BNAs	Toxicity, Pesticides, Metals, TSS, BNAs	Toxicity, Pesticides, Metals, TSS	Toxicity, Pesticides, Metals, TSS
Juanita Creek	Toxicity, Pesticides, Metals, TSS	Toxicity, Pesticides, Metals, TSS	Toxicity, Pesticides, Metals, TSS	Toxicity, Pesticides, Metals, TSS
Lewis Creek	Toxicity, Pesticides, Metals, TSS	No sampling planned	No sampling planned	No sampling planned
Rock Creek Reference Site	Toxicity, Pesticides, Metals, TSS	Toxicity, Pesticides, Metals, TSS	Toxicity, Pesticides, Metals, TSS	Toxicity, Pesticides, Metals, TSS

Dry antecedent conditions were also important to determine an appropriate sampling opportunity and were monitored to determine if an upcoming storm would be suitable. In the spring, when pesticide sales are highest and presumably applications the greatest, a dry period of a few days to a week would give homeowners and lawn-care professionals a chance to apply landscape pesticides. As such, the "ideal" storm would occur after a weekend of good weather. Weather predictions and telemetering devices in the Lyon Creek basin that monitored rainfall and stream flow were used to aid the determination of when to mobilize and initiate sampling.

Samples for toxicity, metals, base neutral acid extractable (BNA) organics, total organic carbon, and total suspended solids were collected as grab samples. Grab samples for low-level metals analysis were collected in general accordance with Method 1669 techniques (U.S. EPA 1996a). Field personnel approached the sites from a downstream direction to minimize disturbance, and collected samples while facing upstream in the middle of the creek to minimize the introduction of contamination. Low level metals, toxicity, BNA organics, total organic carbon, and total suspended solids were analyzed at the King County Environmental Laboratory.

Samples for most pesticide analyses were collected as discharge-weighted composites (see Appendix B). The composite samples were analyzed for various pesticides at the U.S. Geological Survey National Water Quality Laboratory (NWQL) in Denver, CO., and the Washington State Department of Ecology Manchester Environmental Laboratory, in Manchester, WA. Additionally, grab samples for the analysis of the herbicide glyphosate were also collected and analyzed at North Coast Laboratories, Ltd. in Arcata, California. Glyphosate was analyzed in 1999 only.

Stream hydraulics were measured in two ways. First, flow was measured by field personnel during sampling using a flow meter (e.g., Swoffer flow meter). The flow meter provided a discharge rate that is presented in tables below. Second, the stream level was monitored by continuously recording creek level gauges, which have been permanently installed and monitored by King County or the USGS, depending on the stream. Stream levels during storms are represented in the hydrographs below.

Pesticide data from Lyon and Rock Creeks that were collected in May 1998 as part of the USGS pesticide survey are discussed here to aid interpretation of the toxicity test tests that were conducted at Lyon and Rock Creeks at the same time but have not been reported elsewhere.

Tables 2-2 through 2-6 below outline sample collection and handling conditions for each of the sampling events. Data for hydrographs were not available for Lewis or Rock Creeks.

Table 2-2. May 1998 sample collection and handling conditions.

Collection date:	May 14, 1998
Collection method:	Grab flow-weighted composite of multiple grabs
Creeks sampled / flow:	Lyon Creek / 4.5 cfs Rock Creek (reference) / not recorded
Total rainfall of associated storm:	0.24 inches
Antecedent conditions:	Daily rainfall totals for the 19 days prior to sampling were less than the target storm (approximately 0.25 inches).
Chemical Analyses:	Pesticides
Toxicity tests:	Chronic *Ceriodaphnia dubia* Chronic *Selenastrum capricornutum* Chronic *Lemna minor*

Table 2-3. Spring 1999 sample collection and handling conditions.

Collection date:	June 24, 1999
Collection method:	Flow-weighted composite samples collected for pesticides and total suspended solids. Grab samples collected for toxicity tests, glyphosate, metals, and BNA organics.
Creeks sampled / flow:	Juanita Creek / 110 cfs Lewis Creek / 13.5 cfs Lyon Creek / 54.9 cfs Rock Creek (reference) / 5.2 cfs
Total rainfall of associated storm:	1.22 inches
Antecedent conditions:	Daily rainfall totals for the 17 days prior to sampling were less than the target storm (0.25 inches).
Chemical and Physical Analyses:	Total metals, pesticides, total suspended solids, BNA organics (Lyon only).
Toxicity tests:	Chronic Ceriodaphnia dubia Chronic Selenastrum capricornutum

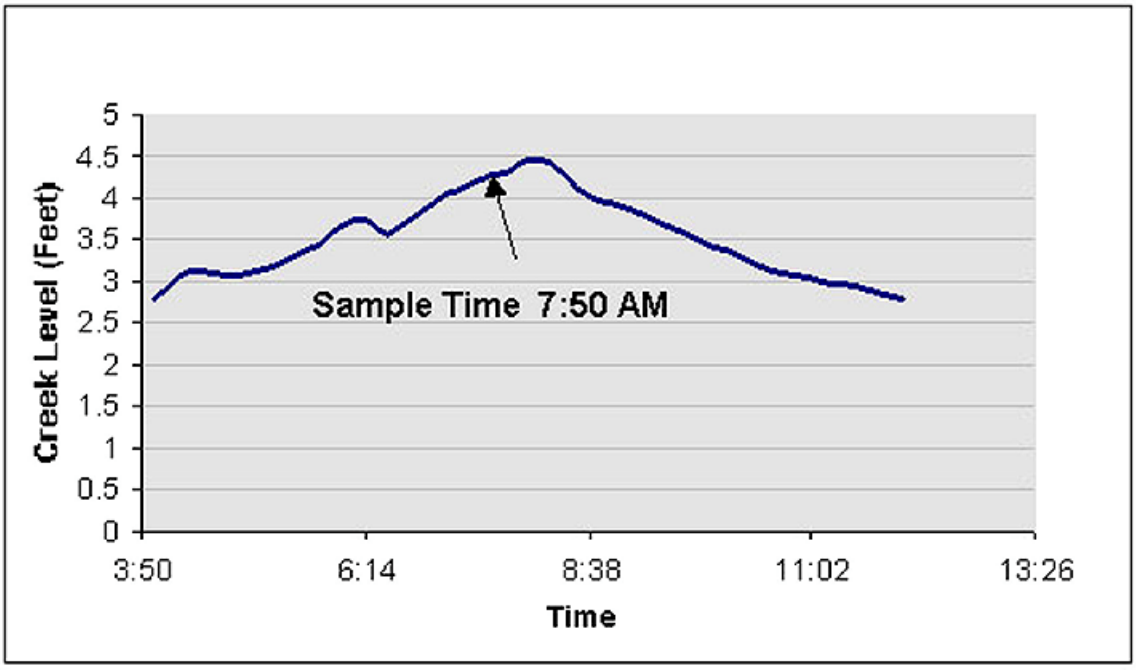

Figure 2. Juanita Creek. June 24, 1999 Sampling Hydrograph

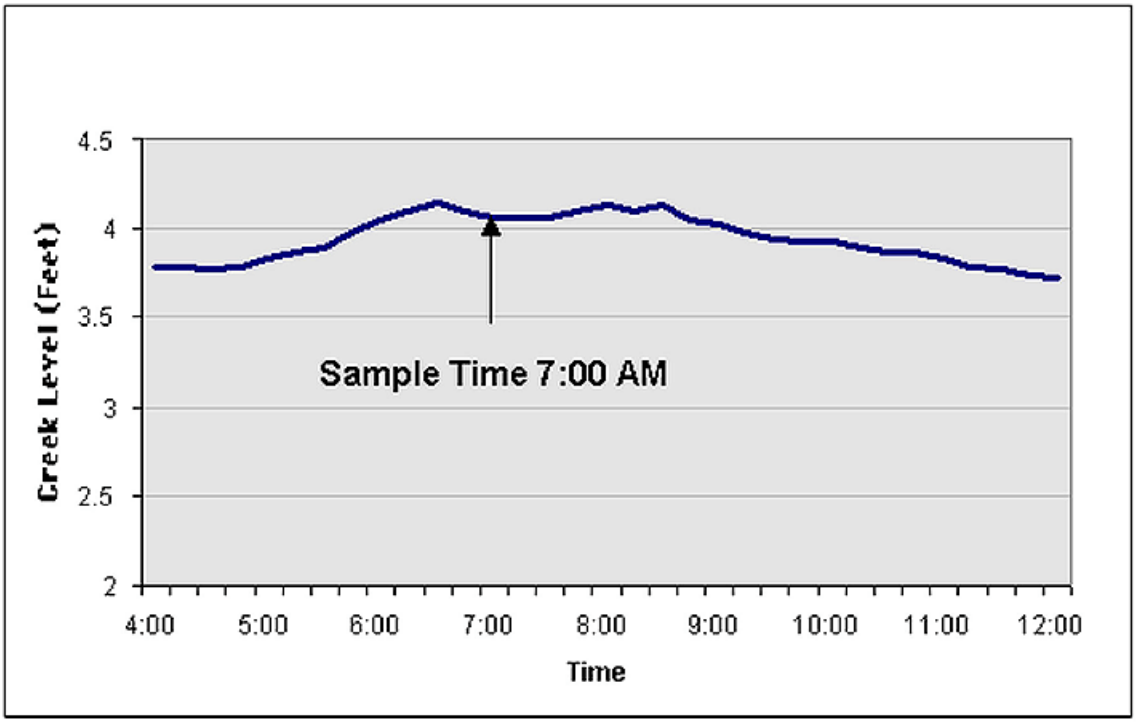

Figure 3. Lyon Creek. June 24, 1999 Sampling Hydrograph

Table 2-4. Summer 1999 sample collection and handling conditions.

Collection date:	August 17, 1999
Collection method:	Flow-weighted composite samples collected for pesticides and total suspended solids. Grab samples collected for toxicity tests, glyphosate, metals, and BNA organics.
Creeks sampled / flow	Juanita Creek / 3.9 cfs Lyon Creek / 1.6 cfs Rock Creek (reference) / 8.96 cfs
Total rainfall of associated storm:	Baseflow
Antecedent conditions:	Baseflow
Chemical and Physical Analyses:	Total metals, pesticides, total suspended solids, BNA organics (Lyon only).
Toxicity tests:	Chronic Ceriodaphnia dubia Chronic Selenastrum capricornutum

Table 2-5. Fall 1999 sample collection and handling conditions.

Collection date:	October 8, 1999
Collection method:	Flow-weighted composite samples collected for pesticides and total suspended solids. Grab samples collected for toxicity tests, glyphosate, and metals.
Creeks sampled / flow:	Juanita Creek / 30 cfs Lyon Creek / 12 cfs Rock Creek (reference) / 16 cfs
Total rainfall of associated storm:	0.59 inches
Antecedent conditions:	Daily rainfall totals for the 84 days prior to sampling were less than the target storm (0.25 inches). No measurable rain fell in the 13 days prior to sampling.
Chemical and Physical Analyses:	Total metals, pesticides, total suspended solids.
Toxicity tests:	Chronic Ceriodaphnia dubia Chronic Selenastrum capricornutum

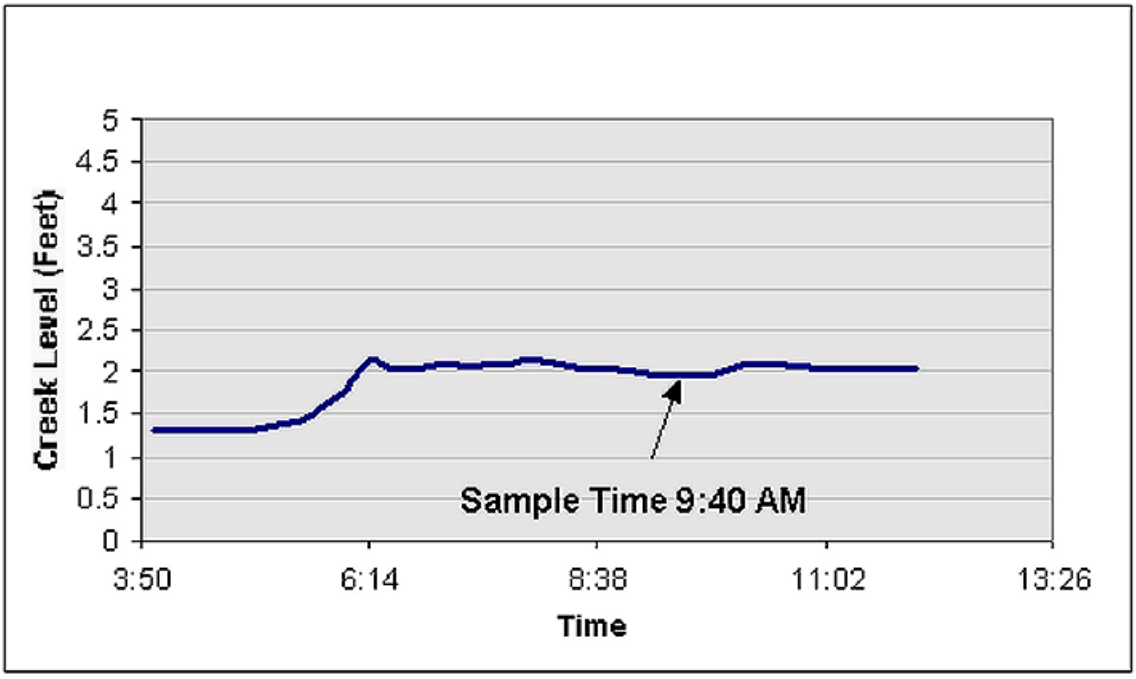

Figure 4. Juanita Creek. October 8, 1999 Sampling Hydrograph

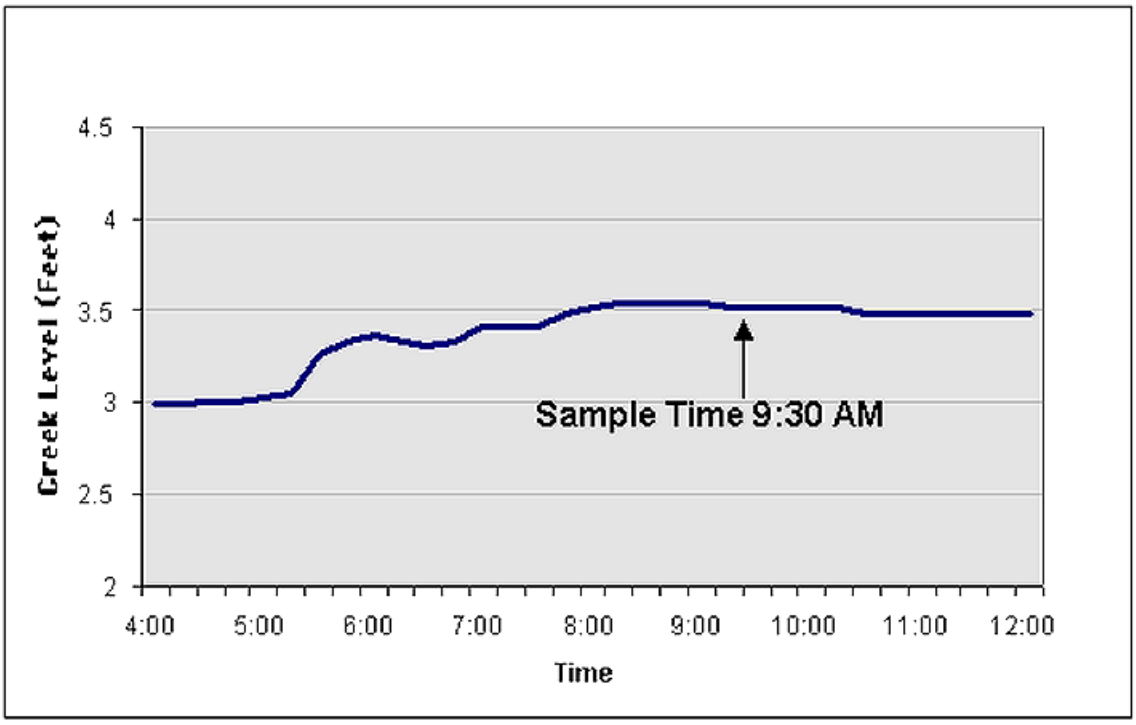

Figure 5. Lyon Creek. October 8, 1999 Sampling Hydrograph

Table 2-6. Late fall 1999 sample collection and handling conditions.

Collection date:	November 16, 1999
Collection method:	Flow-weighted composite samples collected for pesticides and total suspended solids. Grab samples collected for toxicity tests, glyphosate, and metals.
Creeks sampled / flow	Juanita Creek / 7.92 cfs Lyon Creek / 17.0 cfs Rock Creek (reference) / not recorded
Total rainfall of associated storm:	0.63 inches
Antecedent conditions:	None, per study design
Chemical and Physical Analyses:	Total metals, pesticides, total suspended solids.
Toxicity tests:	Chronic Ceriodaphnia dubia Chronic Selenastrum capricornutum

3.4. Toxicity Test Methods

To account for potential differences in species sensitivity, more than one species was selected for the toxicity tests. Given the occurrence of pesticides and herbicides in the

test creeks observed in the USGS pesticide survey, an invertebrate (*Ceriodaphnia dubia*), a green algae (*Selenastrum capricornutum*), and an aquatic vascular plant (*Lemna minor*) were selected. These three organisms reasonably represent the taxa most likely to be affected by pesticides and/or herbicides. However, due to budgetary constraints, *Lemna minor* was only tested on 1998.

In summary, *C. dubia*, a cladoceran, are exposed to the samples in a static renewal system until 60% of surviving control organisms have three broods of offspring. Test results are based on survival and reproduction. For *S. capricornutum*, a unicellular algae species, cells are exposed to the samples in a static system for 96 hours. The response of the population is measured in terms of changes in cell density (cell counts per mL). *Lemna minor*, or duckweed, are exposed to the samples in static renewal system for seven days. The test response is measured in terms of dry weight growth.

Control responses of each species were used to assess the validity of the test. However, test creek sample results were compared to the unfiltered Rock Creek (reference) sample results using appropriate hypothesis tests (U.S. EPA 1994). Furthermore, the samples were tested with and without 0.45 µm filtration to determine if any potential toxicity is associated with particulates.

All toxicity testing was conducted at the King County Environmental Laboratory (KCEL) in Seattle, Washington. The chronic *Ceriodaphnia dubia* and *Selenastrum capricornutum* tests were conducted according to U.S. EPA (1994). Summaries of test conditions for these tests are provided in Tables 2-7 and 2-8.

Table 2-7. Summary of test conditions for the chronic *Ceriodaphnia dubia* toxicity tests.

Test Protocol	*Methods for Measuring the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms* EPA/600/4-91/002, July 1994 [see USEPA, 1999. Errata for the Effluent and Receiving Water Toxicity Testing Manuals. EPA-600/R-98/182].
Test Material	Receiving Water
Test Organisms/age	*Ceriodaphnia* dubia; 24 hrs old
Source of Organisms	In-house culture
Number/Test Chamber	1
Volume/Test Chamber	15 mL
Test Concentrations	100% receiving water (0.45 µm-filtered and unfiltered) and control
Replicates	Ten
Reference Toxicant	Cadmium (as cadmium nitrate)
Test Duration	7 days
Control/Dilution Media	Lake Washington water
Test Chambers	30 mL plastic cups
Lighting	Fluorescent bulbs (50-100 foot candles)
Photoperiod	16 hours light; 8 hours dark
Aeration	None
Feeding	Daily (0.1 mL YCT and 0.05 mL algal suspension at 3.6×10^6 cells/mL)
Renewal	Daily
Temperature	25 ± 1°C
Chemical Data	Dissolved oxygen, temperature, and pH at test initiation and every 24 hours; specific conductivity, hardness, and alkalinity
Effect Measured	Mortality and reproduction
Test Acceptability	Control mortality ≤80%, an average of 15 neonates per surviving adult in the control, ≥60 percent of the surviving control organisms producing three broods.

Table 2-8. Summary of test conditions for the chronic *Selenastrum capricornutum* toxicity tests.

Test Protocol	*Methods for Measuring the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms* EPA/600/4-91/002, July 1994 [see USEPA, 1999. Errata for the Effluent and Receiving Water Toxicity Testing Manuals. EPA-600/R-98/182].
Test Material	Receiving water
Test Organisms/Age	*S. capricornutum*; 4-7 days from culture renewal
Source	In-house culture, originally obtained from the American Type Culture Collection (12301 Park Lawn Dr., Rockville, Maryland 20852)
Number/Container	10,000 cells/mL at test initiation
Volume/Container	50 mL
Test Concentrations	100% receiving water (0.45 µm-filtered and unfiltered) and control
Replicates	Four
Reference Toxicant	Sodium chloride
Test Duration	96 hours
Control/Dilution Media	Algal assay medium
Test Chambers	125 mL glass Erlenmeyer flasks
Lighting	86 ± 8.6 µE/m ^{2} /s (400 ± 40 ft-c or 4300 ± 430 lux)
Photoperiod	Continuous illumination
Shaking Rate	Twice daily, by hand
Temperature	25 ± 1° C
Chemical Data	Temperature at initiation and every 24 hours; pH at initiation and termination of test
Effect Measured	Cell growth
Test Acceptability	Control ≥ 1.0 x 10^6 cells/mL

The chronic *Lemna minor* test was conducted according to ASTM (1988) modified to be conducted with a static-renewal procedure and use of Hoagland's medium at 10% of full strength. Full strength Hoagland's medium has a pH value of 5.4. Hoagland's medium was used at 10% of full strength to permit adjustment of pH to ≥ 7.0 without precipitation of medium components and to reduce the possibility of masking the chemical nature of the sample with medium components. It was necessary to add nutrients to all samples so that nutrient-limited growth was not confused with toxicity. The nutrient medium was diluted as much as possible to minimize changing the sample matrix. Because the medium was diluted, renewals were performed to insure an adequate level of nutrients for plant growth. A summary of test conditions for this test is provided in Table 2-9.

Table 2-9. Summary of test conditions for the chronic *Lemna minor* toxicity tests.

Test Protocol	*Proposed new standard guide for conducting static and acute toxicity tests with duckweed.* Draft #7. ASTM 1988.
Test Material	Receiving water
Test Organisms/Age	*L. minor*; 3-frond
Source	Illinois State Water Survey, November 11, 1988
Number/Container	3, 3-frond plants
Volume/Container	50 mL
Test Concentrations	100% receiving water (0.45 µm-filtered and unfiltered) and control
Replicates	Four
Reference Toxicant	Sodium chloride
Test Duration	7 days
Control/Dilution Media	Hoagland's medium at 10% full strength
Test Chambers	100 mL glass beakers
Lighting	86 ± 8.6 µE/m^2 /s (400 ± 40 ft-c or 4300 ± 430 lux)
Photoperiod	Continuous illumination
Renewal	Daily
Temperature	25 ± 1° C
Chemical Data	Temperature and pH at initiation and every 24 hours
Effect Measured	Growth
Test Acceptability	Growth (dry weight) of control > 3 times the dry weight of the inoculum

3.5. Chemical Methods

3.5.1. Collection Methods

Samples to be analyzed for pesticides and pesticide transformation products, total metals, base/neutral/acid (BNA) extractable organic compounds, hardness, and total suspended solids (TSS) were collected concurrently with the toxicity test samples. Sample collection and processing methods for the pesticides and pesticide transformation products are described in detail in Appendix B.

Samples for metals analyses were only collected in 1999 following the same general guidelines (U.S. EPA 1996a) and conditions as for the toxicity test sample collection described in Section 2.3; no metals samples were collected in 1998. Two-person sampling teams were employed using the "dirty hands / clean hands" sampling technique described in U.S. EPA (1996a) for collection of all samples for metals analysis. All metals sample bottles were pre-cleaned and double bagged.

Grab samples for analysis of TSS, hardness, and glyphosate samples were collected concurrently with every event in 1999. Base/neutral/acid (BNA) extractable organic compounds were also measured in Lyon Creek samples during the spring and summer 1999 sample events.

3.5.2. Analytical Methods

Detailed methods for pesticide analyses (excluding glyphosate) conducted by the USGS and DOE are described in Appendix B. The pesticides measured by the USGS laboratory include 26 herbicides, 18 insecticides, and 3 transformation products for a total of 47 analytes. The pesticides measured by the DOE Manchester Laboratory include 12 fungicides, 60 herbicides, 58 insecticides, and 12 transformation products, for a total of 142 analytes. Thirty-five of the analytes measured by USGS were also measured by DOE; therefore, 154 total analytes were analyzed by both laboratories. The USGS method described in Appendix B requires 0.7-µm filtration of the samples prior to analysis; the DOE method is conducted on the raw sample with no filtration.

Total metals, hardness, and TSS were analyzed at the King County Environmental Laboratory. The samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) for aluminum, antimony, arsenic, barium, beryllium, cadmium, chromium, cobalt, copper, lead, molybdenum, nickel, selenium, silver, thallium, vanadium and zinc. Mercury was determined by cold-vapor atomic absorbance. Hardness was calculated from the concentrations of calcium and magnesium as determined by ICP; and TSS was determined by Method CV SM2540-D. BNA analyses were performed by method SW846-8270 using gas chromatography-mass spectrometer techniques. Finally, glyphosate was analyzed at North Coast Laboratories in Arcata, California, using EPA Method 547.